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Date: February 19, 2002

By: 

Kay L. Gaviglio

**PATENT**  
**Docket No. GC567C1**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of	)	
	)	
Huaming Wang	)	Group Art Unit: - -
	)	
Serial No.: Cont of 09/218,702	)	Examiner: - -
	)	
Filed: Herewith	)	
	)	
For: NOVEL PHENOL OXIDIZING	)	
ENZYMES	)	

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to reviewing the above-identified application on the merits, please enter the following amendment.

**IN THE SPECIFICATION.**

Please place the sequence listing after the Abstract.

**IN THE CLAIMS.**

Please cancel claims 1, 4 - 7, 9, 10, 21, 22 and 38.

Please enter the following amended claims. A marked-up version of each amended claim is appended hereto as Appendix I. Additionally, Applicant has attached a copy of the status of the claims after entry of this amendment as Appendix II.

2.(Once amended) The phenol oxidizing enzyme of Claim 40 wherein said *Stachybotrys* includes *S. parvispora*, *S. chartarum*, *S. kampalensis*, *S. theobromae*, *S. bisbyi*, *S. cylindrospora*, *S. dichroa*, *S. oenanthos* or *S. nilagerica*.

3.(Once amended) An isolated phenol oxidizing enzyme having the amino acid sequence disclosed in SEQ ID NO: 2.

12.(Once amended) A host cell comprising the expression vector of Claim 11.

17.(Once amended) The host cell of Claim 12 wherein said host is a bacterium.

19. (Once amended) A method for producing a phenol oxidizing enzyme in a host cell comprising the steps of:

- (a) obtaining a host cell transformed with a polynucleotide encoding a phenol oxidizing enzyme wherein the amino acid sequence of said enzyme has at least 65% identity to the amino acid sequence of SEQ ID NO: 2;
- (b) growing said host cell under conditions suitable for the production of said phenol oxidizing enzyme; and
- (c) recovering said phenol oxidizing enzyme produced.

20.(Once amended) The method of Claim 44 wherein said *Stachybotrys* includes *S. parvispora*, *S. chartarum*, *S. kampalensis*, *S. theobromae*, *S. bisbyi*, *S. cylindrospora*, *S. dichroa*, *S. oenanthos* or *S. nilagerica*.

23.(Once amended) The method of Claim 19 wherein said host cell is selected from the group consisting of filamentous fungus, yeast and bacteria.

30. (Once amended) A method for producing a host cell comprising a polynucleotide encoding a phenol oxidizing enzyme wherein the amino acid sequence of said enzyme has at least 65% identity to the amino acid sequence of SEQ ID NO: 2 comprising the steps of:

- (a) obtaining a polynucleotide encoding said phenol oxidizing enzyme;
- (b) introducing said polynucleotide into said host cell; and

- (c) growing said host cell under conditions suitable for the production of said phenol oxidizing enzyme.

31.(Once amended) The method of Claim 30 wherein said host cell is selected from the group consisting of filamentous fungus, yeast and bacteria.

Please add the following new claims

39. A phenol oxidizing enzyme having at least 70% identity to the amino acid sequence disclosed in SEQ ID NO: 2 wherein said enzyme is capable of modifying the color associated with dyes or a colored compound.

40. The phenol oxidizing enzyme of Claim 39, wherein the enzyme has at least 80% identity to the amino acid sequence of SEQ ID NO: 2.

41. The phenol oxidizing enzyme of Claim 40, wherein said enzyme has at least 90% identity to the amino acid sequence disclosed in SEQ ID NO: 2.

42. A phenol oxidizing enzyme having at least 95% identity to the amino acid sequence of SEQ ID NO: 2.

43. The phenol oxidizing enzyme of Claim 39, wherein the enzyme is obtained from *Stachybotrys*.

44. The method of Claim 19, wherein the phenol oxidizing enzyme is obtained from *Stachybotrys*.

45. An expression vector comprising a polynucleotide encoding the enzyme of Claim 42.

**REMARKS**

With entry of the instant amendment claims 2, 3, 8, 11 - 20, 23 - 37 and 39 - 45 are pending. Claims 1, 4 - 7, 9, 10, 21, 22, and 38 have been canceled. Claims 2, 3, 12, 17, 19, 20, 23, 30 and 31 have been amended, and claims 39 - 45 are new. No new matter has been introduced by the instant amendment.

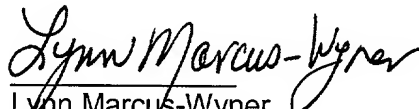
The dependency of claims 2, 12, 17 and 20 has been changed. Claim 3 has been rewritten as an independent claim. Minor wording changes have been made to claims 19 and 30 to more clearly define the invention. Claims 23 and 31 have been amended to include Markush language. New claims 39 - 42 are directed to a phenol oxidizing enzyme having at least 70%, 80%, 90% and 95% identity, respectively to the amino acid sequence of SEQ ID NO: 2. Support is found in the original claims and at page 4, lines 7 - 12 of the specification. Additionally, claims 39 - 42 recite that the phenol oxidizing enzyme is capable of modifying the color associated with dyes or a colored compound. Support for this language is found at page 6, line 22 through page 7, line 5. Claims 43 and 44 recite that the phenol oxidizing enzyme is obtained from a *Stachybotrys* species, and support is found in the original claims. Claim 45 is directed to an expression vector comprising a polynucleotide encoding the enzyme of claim 42.

Applicant has also amended the specification to include the sequence listing after the abstract.

Applicant contends all claims are in form for allowance and allowance of the pending claims is kindly solicited.

Respectfully submitted,

Date: February 19, 2002

  
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Enclosure - Appendix I - Marked-up version of amended claims  
Appendix II - Copy of claim status.

**APPENDIX I – MARKED-UP VERSION OF THE CLAIMS**

2.(Once amended) The phenol oxidizing enzyme of **[Claim 1] Claim 40** wherein said *Stachybotrys* includes *S. parvispora*, *S. chartarum*, *S. kampalensis*, *S. theobromae*, *S. bisbyi*, *S. cylindrospora*, *S. dichroa*, *S. oenanthos* **[and] or** *S. nilagerica*.

3.(Once amended) **[The phenol oxidizing enzyme of claim 1] An isolated phenol oxidizing enzyme** having the amino acid sequence **[as]** disclosed in SEQ ID NO: 2.

12.(Once amended) A host cell comprising the expression vector of **[Claim 9, Claim 10, or] Claim 11**.

17.(Once amended) The host cell of **[Claim 13] Claim 12** wherein said host is a bacterium.

19. (Once amended) A method for producing a phenol oxidizing enzyme **[obtainable from *Stachybotrys*]** in a host cell comprising the steps of:

- (a) obtaining a host cell **transformed with** **[comprising]** a polynucleotide encoding **[said phenol oxidizing enzyme obtainable from *Stachybotrys* wherein said enzyme ] a phenol oxidizing enzyme wherein the amino acid sequence of said enzyme** has at least 65% identity to the amino acid sequence **[disclosed in] of** SEQ ID NO: 2;
- (b) growing said host cell under conditions suitable for the production of said phenol oxidizing enzyme; and
- (c) **[optionally]** recovering said phenol oxidizing enzyme produced.

20.(Once amended) The method of **[Claim 19] Claim 44** wherein said *Stachybotrys* includes *S. parvispora*, *S. chartarum*, *S. kampalensis*, *S. theobromae*, *S. bisbyi*, *S. cylindrospora*, *S. dichroa*, *S. oenanthos* **[and] or** *S. nilagerica*.

23.(Once amended) The method of Claim 19 wherein said host cell **[includes] is selected from the group consisting of** filamentous fungus, yeast and bacteria.

30. (Once amended) A method for producing a host cell comprising a polynucleotide encoding a phenol oxidizing enzyme **[obtainable from Stachybotrys and having]** **wherein the amino acid sequence of said enzyme has** at least 65% identity to the amino acid sequence **[disclosed in] of** SEQ ID NO: 2 comprising the steps of:

- (a) obtaining a polynucleotide encoding said phenol oxidizing enzyme;
- (b) introducing said polynucleotide into said host cell; and
- (c) growing said host cell under conditions suitable for the production of said phenol oxidizing enzyme.

31. (Once amended) The method of Claim 30 wherein said host cell **[includes] is** **selected from the group consisting of** filamentous fungus, yeast and bacteria.

**APPENDIX II – STATUS OF CLAIMS**

1. Canceled

2.(Once amended) The phenol oxidizing enzyme of Claim 40 wherein said *Stachybotrys* includes *S. parvispora*, *S. chartarum*, *S. kampalensis*, *S. theobromae*, *S. bisbyi*, *S. cylindrospora*, *S. dichroa*, *S. oenanthae* or *S. nilagerica*.

3.(Once amended) An isolated phenol oxidizing enzyme having the amino acid sequence disclosed in SEQ ID NO: 2.

4. – 7. Canceled

8. An isolated polynucleotide capable of hybridizing to the polynucleotide having the sequence as shown in SEQ ID NO: 1 under conditions of high stringency.

9. Canceled

10. Canceled

11. An expression vector comprising the polynucleotide of claim 8.

12.(Once amended) A host cell comprising the expression vector of Claim 11.

13. The host cell of Claim 12 that is a filamentous fungus.

14. The host cell of Claim 13 wherein said filamentous fungus includes *Aspergillus* species, *Trichoderma* species and *Mucor* species.

15. The host cell of claim 13 that is a yeast.

16. The host cell of Claim 15 wherein said yeast includes *Saccharomyces*, *Pichia*, *Schizosaccharomyces*, *Hansenula*, *Kluyveromyces* and *Yarrowia* species.

17.(Once amended) The host cell of Claim 12 wherein said host is a bacterium.

18. The host cell of Claim 17 wherein said bacterium includes *Bacillus* and *Escherichia* species.

19. (Once amended) A method for producing a phenol oxidizing enzyme in a host cell comprising the steps of:

- (a) obtaining a host cell transformed with a polynucleotide encoding a phenol oxidizing enzyme wherein the amino acid sequence of said enzyme has at least 65% identity to the amino acid sequence of SEQ ID NO: 2;
- (b) growing said host cell under conditions suitable for the production of said phenol oxidizing enzyme; and
- (c) recovering said phenol oxidizing enzyme produced.

20.(Once amended) The method of Claim 44 wherein said *Stachybotrys* includes *S. parvispora*, *S. chartarum*, *S. kampalensis*, *S. theobromae*, *S. bisbyi*, *S. cylindrospora*, *S. dichroa*, *S. oenantes* or *S. nilagerica*.

21, Canceled

22. Canceled

23.(Once amended) The method of Claim 19 wherein said host cell is selected from the group consisting of filamentous fungus, yeast and bacteria.

24. The method of Claim 23 wherein said yeast includes *Saccharomyces*, *Pichia*, *Schizosaccharomyces*, *Hansenula*, *Kluyveromyces* and *Yarrowia* species.

25. The method of Claim 23 wherein said filamentous fungus includes *Aspergillus* species, *Trichoderma* species and *Mucor* species.

26. The method of Claim 25 wherein said filamentous fungus is a species of *Aspergillus*.

27. The method of Claim 26 wherein said filamentous fungus is *Aspergillus niger* var. *awamori*.

28. The method of Claim 23 wherein said filamentous fungus is a species of *Trichoderma*.

29. The method of Claim 28 wherein said *Trichoderma* species is *Trichoderma reesei*.

30. (Once amended) A method for producing a host cell comprising a polynucleotide encoding a phenol oxidizing enzyme wherein the amino acid sequence of said enzyme has at least 65% identity to the amino acid sequence of SEQ ID NO: 2 comprising the steps of:

- (a) obtaining a polynucleotide encoding said phenol oxidizing enzyme;
- (b) introducing said polynucleotide into said host cell; and
- (c) growing said host cell under conditions suitable for the production of said phenol oxidizing enzyme.

31.(Once amended) The method of Claim 30 wherein said host cell is selected from the group consisting of filamentous fungus, yeast and bacteria.

32. The method of Claim 31 wherein said filamentous fungus includes *Aspergillus* species, *Trichoderma* species and *Mucor* species.

33. The method of Claim 32 wherein said *Aspergillus* species is *Aspergillus niger* var. *awamori*.

34. The method of Claim 32 wherein said *Trichoderma* species is *Trichoderma reesei*.

35. The method of Claim 31 wherein said yeast includes *Saccharomyces* species.

36. The method of Claim 35 wherein said *Saccharomyces* species is *Saccharomyces cerevisiae*.

37. The method of Claim 30 wherein said polynucleotide has at least 65% identity to the nucleic acid shown in SEQ ID NO: 1 or SEQ ID NO: 3.

38. Canceled

39.(New) A phenol oxidizing enzyme having at least 70% identity to the amino acid sequence disclosed in SEQ ID NO: 2 wherein said enzyme is capable of modifying the color associated with dyes or a colored compound.

40.(New) The phenol oxidizing enzyme of Claim 39, wherein the enzyme has at least 80% identity to the amino acid sequence of SEQ ID NO: 2.

41.(New) The phenol oxidizing enzyme of Claim 40, wherein said enzyme has at least 90% identity to the amino acid sequence disclosed in SEQ ID NO: 2.

42.(New) A phenol oxidizing enzyme having at least 95% identity to the amino acid sequence of SEQ ID NO: 2.

43.(New) The phenol oxidizing enzyme of Claim 39, wherein the enzyme is obtained from *Stachybotrys*.

44.(New) The method of Claim 19, wherein the phenol oxidizing enzyme is obtained from *Stachybotrys*.

45.(New) An expression vector comprising a polynucleotide encoding the enzyme of Claim 42.